Aventis Position on Follow Up with Individuals Alleging Allergic Reactions to StarLink™ Corn Ingestion

Introduction

Although the report of the EPA's November 28, 2000 FIFRA Scientific Advisory Panel concluded that there was a low probability that the likely levels of the Cry9C protein in the US diet were sufficient to cause significant allergic reactions in the exposed population, it also recommended follow up with individuals alleging adverse effects after consumption of foods possibly containing StarLink™ corn. During the March 1 meeting between representatives from Aventis, FDA, CDC and EPA, you welcomed the suggestions of Aventis to ensure scientific rigor in this investigative process. As previously stated, our desire is for the CDC/FDA to develop a robust scientific testing paradigm and avoid raising unnecessary concerns with respect to the safety of the US food supply. To assist in attaining these goals, Aventis offers the following suggested process.

Medical Questionnaire/History

The December, 2000 SAP report recommended that the CDC/FDA follow-up include completion of medical questionnaires, and recognized the limitations in this type of investigation:

> "In dealing with self-reported reactions, it is well known that patient history often can not be confirmed. In fact, less than 40% of reported food allergic reactions can be confirmed when patients are subjected to double-blind placebo-controlled food challenges [DBPCFC]… As noted above, the majority of reported adverse reactions to foods can not be validated when patients are subjected to DBPCFCs. Perceived food allergy is probably 10 times greater than actual food allergy." (FIFRA Scientific Advisory Panel, 1)

Aventis wishes to emphasize the limitations of patient recall and cites Dr. Hugh A. Sampson, SAP member, who wrote:

> "The medical history relies on the patient's or his or her parents' recollection of events surrounding the development of symptoms and generally must be considered subjective at best. Loveless was among the first to point out the frequent inaccuracy of patient history, and May repeatedly warned of the power of the mind to misperceive the etiology of an ailment. A review of several published series in which double-blind, placebo-controlled, food challenges (DBPCFCs) were used to establish the diagnosis of food allergy reveals that only 40% of patients' histories of food-induced allergic reactions can be verified."(Sampson, 2)

In reality, food allergy affects only 1-2% of adults and 6-8% of children in the United States, according to SAP members Hugh Sampson, Dean Metcalfe and other well known experts. (Sampson, 3; Metcalfe, 4 Therefore, Aventis expects that the individuals alleging adverse food allergic reactions to products potentially containing StarLink™ corn will not be clinically allergic.

The SAP report stated that a systematic approach should be followed in an investigative process in order to come to the correct diagnosis of food allergy.

> "The clinical history must address several issues:
> (1) Description of the food eliciting the reaction.
> (2) Description of symptoms elicited.
> (3) Quantity of food ingested.
> (4) Timing of reaction.
> (5) Whether the individual has eaten the food before.
> (6) Is the reaction reproducible."(FIFRA Scientific Advisory Panel, 1)

Aventis agrees with the SAP experts that these elements must be included in a rigorous scientific investigation, but also suggests that each of the individuals alleging allergic reactions to presumed ingestion of corn products containing StarLink™ corn undergo a thorough physical examination according to the advice of Dr. Wesley Burks, Professor of Pediatrics in the Division of Pediatric Allergy and Immunology at the Arkansas Children's Hospital Research Institute, Hugh Sampson and Dean Metcalfe (SAP members) on diagnosing adverse food reactions.

> "As with all medical disorders, the diagnostic approach to the patient with a suspected adverse food reaction begins with the medical history and physical examination…The true value of the medical history is largely dependent on the patient's recollection of symptoms and the physician's ability to differentiate disorders provoked by food hypersensitivity and other possible etiologies". (Burks, 5)
>
> "The diagnostic approach to suspected adverse food reactions begins with the medical history and physical examination."(Sampson, 2)
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> "The physical examination should emphasize the cutaneous, respiratory, and gastrointestinal systems". (Bock, et al., 6)

Additional elements of a clinical history recommended by Dr. Burks and strongly supported by Aventis include determining:

1. the length of time between the ingestion of the food and the development of symptoms,
2. whether or not the individual has experienced similar symptoms upon previous ingestion of the same food,
3. the length of time since the last reaction, and
4. if any other factors, such as exercise are necessary for the experience of the reaction. (Some allergic reactions are exercise-induced.) (Burks, 5)

In addition, Aventis believes the clinical history of those alleging a reaction to foods presumed to contain StarLink corn should include other allergies the person might have, as well as what other foods were consumed (and the quantity of each) and what activities were performed the day the alleged incident occurred.

Aventis strongly agrees with the testimony to the EPA's November 28, 2000 SAP of Dr. Carol Rubin, of the CDC that the

> "Review of adverse event reports will help to describe the characteristics of the reported cases, but will not determine if the reported symptoms were due to consumption of products containing StarLink™ corn…" (Rubin, 7)

Therefore, Aventis believes strongly that the collection of the adverse effects report, medical histories and questionnaires and physical exams should be only one step in this StarLink™ corn investigative process.

Analysis of Food Samples

Aventis strongly concurs with the testimony of Dr. Larry Williams at the November 2000 FIFRA SAP meeting that:

> "the first step in rigorously investigating allegations of adverse reactions after consumption of a corn product alleged to contain StarLink™ corn is to prove that the protein is present in the food as consumed." (Williams, 8)

Aventis strongly supports CDC efforts to collect, preserve and analyze samples of food from each of the individuals alleging allergic reactions after ingestion of corn products allegedly containing the Cry9C protein from StarLink™ corn. Since the November SAP meeting, protein extraction methods have been developed and validated for the Cry9C protein in many corn food matrices, by both Aventis and independent contractors, for example EnviroLogix. Confirmation of any positive detection of Cry9C in the food products should be made by an independent qualified laboratory with experience in protein extraction and analysis.

Aventis believes that it is absolutely essential that there be valid evidence that the individuals alleging allergic reactions to the consumption of corn products actually were exposed to the Cry9C protein. Without proof that the Cry9C protein was even consumed, any suggestive medical history, questionnaire data or physical examination findings, positive ELISA positive results will be equivocal at best. The investigation will be subject to questions about false positives, cross reactivity of the detection method to other molecules and predictive value of the ELISA data.

Analysis of Blood Samples for Cry9C-Specific Antibodies

The FIFRA SAP report also advised that blood samples be taken from the individuals alleging allergic reactions to the ingestion of foods supposedly containing StarLink™ corn for sera testing for the presence of specific antibodies to StarLink™ corn. Aventis agrees that most well-characterized allergic reactions to foods are IgE-mediated hypersensitivity reactions (FIFRA Scientific Advisory Panel, 1; Taylor, 9). Aventis also

recognizes the diagnostic limitations of assays designed to measure IgE specific to the Cry9C protein as expressed by the FIFRA SAP.

> "In dealing with IgE-mediated reactions, the presence of positive skin tests or demonstration of food-specific IgE antibodies in the serum is suggestive of clinical reactivity. About 30% - 40% of patients with IgE antibody to a food will ultimately react in a DBPCFC…Ultimately the use of the DBPCFC can determine clinical reactivity." (FIFRA Scientific Advisory Panel, 1)

ELISA - Optimization

Aventis suggests that with each step of the development of an enzyme linked immunosorbent assay (ELISA) to quantify immunoglobulins against a protein, it is essential to optimize the assay. Variations in the assays being developed by the FDA and the independent laboratory (Burleson Research Technology, BRT, of Raleigh, NC) may exist in one or all of the following, affecting the comparability of results and assay sensitivity and specificity:

1. the concentration of Cry9C protein used to coat the wells of the plates, which will affect the antigen-antibody reaction ad the potential 'signal' generated,
2. the coating buffers used to optimize antigen binding for optimal antigen-antibody reaction,
3. cross titration of primary and secondary antibody to optimize the detection of Cry9C-specific immunoglobulins in the sera,
4. the selection of different substrates (TMB, Blue-Phos, etc.) and detection systems, which will affect detection,
5. the selection of different blocking buffers to reduce the background reactivity in human blood and increase assay detection limits,
6. the incubation times and temperatures at which the sera contacts the plates, affecting the detection limits of the assay.

ELISA – Standard Curve

In the development of Cry9C-specific ELISAs, Aventis strongly suggests the inclusion of the generation of a standard curve. According to Yunginger, et al.:

> "If the solid-phase allergen is present in excess and has the capacity to bind the IgE antibodies quantitatively over the whole range of concentrations, this permits demonstration of parallelism between the different samples and the calibrator curve even above this range."(Yunginger, et al., 10)

ELISA – Control Sera and Baseline Reactivity

Yet even with these measures to ensure that the assays will discriminate any Cry9C antibody Aventis strongly emphasizes that without human serum with antibodies to the Cry9C protein in StarLink™ corn, an ELISA method to detect Cry9C-specific human IgG and IgE antibodies is limited because there can be no "positive control" used in the validation of the method. If the ELISA is too sensitive, it will yield false positives by indicating the presence of Cry9C-specific antibodies where none exist. If it is not sensitive enough, it will yield false negatives.

Therefore, 60-70% of patients with IgE specific to Cry9C will NOT ultimately have clinical food allergy to the Cry9C protein in StarLink™ corn. To help increase the specificity of the ELISA assays under development (by the FDA and an independent laboratory, Burleson Research Technology) Aventis strongly recommends that the following sera be used as the development and validation of the assays:

1. sera from well-characterized atopic and non-atopic individuals (already provided to the FDA by Dr. Susan Hefle of the University of Nebraska),
2. sera from bona fide corn allergic individual(s) (potentially to be provided by Dr. Sam Lehrer of Tulane University on behalf of consortium of companies funding corn allergy research),
3. sera collected from individuals prior to the 1998 introduction of StarLink™ corn or sera from individuals living in countries where StarLink™ corn was never grown nor sold.

ELISA – Qualifications of Laboratory Performing Assays

Any ELISA results obtained from individuals exposed to Cry9C will always be subject to question because of the lack of positive controls and the probability of false positives and negatives. Aventis believes it is in the best interests of all parties to have reproducible, scientifically defensible assays with good sensitivity to detect true antibodies against the Cry9C protein and good specificity to distinguish between Cry9C-specific antibodies and non-specific reactions. The FDA-developed ELISA and the BRT-developed ELISA need to be validated to ensure reproducibility (i.e., to make sure that the same samples run using the exact same assay will be reproduced in a separate qualified laboratory). Recommended interassay coefficient of variation in IgE antibody assays should not exceed 15%, according to the National Committee for Clinical Laboratory Standards. (Yunginger et al., 10) Aventis believes that the two assays should be specific, sensitive and reproducible.

Aventis strongly believes that these ELISAs and tests for reproducibility and validation be performed in a laboratory, such as Burleson Research Technology, that meets the standards of The Clinical Laboratory Improvement Amendments of 1988 (also known as CLIA). Dr. John Yunginger, of the Mayo Clinic, and colleagues explained the CLIA standards in a recent publication.

> "These standards include requirements for trained supervisory and testing personnel, record keeping and instrument maintenance, daily quality control practices, result reporting, and laboratory inspection and accreditation." (Yunginger, et al., 10)

Interpretation of ELISA – Defining a "Positive" Result

Statistical calculations reveal that running 100 non- StarLink™ corn-exposed sera through the Cry9C-specific ELISAs will have a power of 0.9 (out of a possible 1.0) in detecting any background reactivity in the Cry9C-specific ELISAs in non-exposed individuals, indicating the value of this sample size.

Aventis also strongly recommends that the distribution, mean, and standard deviation of these control sera samples be determined in order to determine what level of reactivity will be defined as a positive ELISA. It is the conviction of Aventis that no reactivity in the Cry9C-specific IgG or IgE ELISAs that is less than three (3) times the standard deviation of the combined control sera be considered positive.

Regarding the interpretation of ELISA results on individuals alleging adverse reactions to presumed StarLink™ corn ingestion, Aventis disagrees with the CDC definition of a probable case as stated in the December SAP report:

> "The CDC considered cases 'probable' if the patient was treated with corticosteroids or antihistamines and/or was diagnosed by a physician as a probable food allergy. The CDC would consider a case 'confirmed' if one of the probable cases had evidence of IgG or IgE antibody to the Cry9C protein."(FIFRA Scientific Advisory Panel, 1)

The high rate of false positives in the ELISA limits any scientific conclusions that might be drawn from its results.

Since most food allergy is IgE-mediated (FIFRA Scientific Advisory Panel, 1; Taylor, 9) Aventis believes that defining a "confirmed case" on the presence of Cry9C-specific IgG is not appropriate. A 'positive' ELISA result for Cry9C-specific IgG does not necessarily imply an induction of a Cry9C-specific IgE response, according to Steve Taylor, co-director of the Food Allergy Research and Resource Program at the University of Nebraska5. Dr. Taylor notes that:

> "The allergenic determinants on a protein that stimulate IgE production may be quite different from the determinants that stimulate IgG production. Also, it must be stressed that only a few individuals will develop allergen-specific IgE and an allergic response after exposure to a potentially allergenic food protein. Only those individuals who have inherited the genetic predisposition to produce IgE antibodies in response to allergen stimulation are likely to display such responses, and then only if they are exposed under suitable conditions." (Taylor, 9)

Therefore, Aventis concurs that basing a definition of a "probable" case of food allergy on medical history dependent upon the patient's recall or the physician's conclusion at the time of treatment alone is not robust nor does it meet stringent scientific standards.

Furthermore, Aventis agrees with the FIFRA SAP that the presence of IgG or IgE antibodies to Cry9C is not sufficient to label a case as "confirmed" as the CDC had recommended. Aventis agrees with the SAP conclusion that the medical histories plus evidence of IgE antibody to Cry9C are insufficient to conclude a clinical food allergy diagnosis.

> "Instead, the Panel believed that given the histories provided plus evidence of IgE antibody to Cry9C, clinical reactivity could be considered 'very likely'… The Panel concluded that it would not be possible to answer the question with absolute certainty of whether StarLink™ can induce an allergic reaction without challenging at least some of these patients. The DBPCFC done in a center skilled in the technique is a safe

> procedure and is considered standard practice in the diagnosis of food allergy."(FIFRA Scientific Advisory Panel, 1)

Follow up with DBPCFC on Positive ELISA Results

The results of these individuals' ELISA for the presence of Cry9C-specific IgE and IgG will not allow definitive allergenicity conclusions. Food allergy experts agree that an ELISA test alone is a poor predictor of human clinical allergic reactivity. Larry Williams, a medical doctor and Associate Professor of Pediatrics in the Division of Allergy and Immunology at Duke University School of Medicine, told the November 28, 2000 SAP that the presence of antibodies to the Cry9C protein in StarLink™ corn detected in human serum is not sufficient to cause an allergic reaction. The SAP report agreed with Dr. Williams and concluded that only 30-40% of individuals with antibodies to a protein in food would have a clinical food allergy, and 60 – 70% of individuals with a positive ELISA test for Cry9C-specific IgG and/or IgE would not have a clinical food allergy.

Aventis strongly concurs with the FIFRA SAP report and with the majority of food allergy experts that the tests for the presence of food-specific antibodies must be followed up with a food challenge to diagnose clinical food allergy.(FIFRA Scientific Advisory Panel, 1; Taylor,9; Hefle,11)

> "DBPCFCs represent the most objective diagnostic approach to establish a cause-and-effect relationship between ingestion of a food and an allergic reaction…Challenge trials establish a cause-and-effect relationship between ingestion of the food and the onset of symptoms in a sensitized individual". (Hefle, 11)

Dr. Hefle believes that immunoassays alone are "insufficient to establish a cause-and-effect relationship because false positives, and to a lesser extent, false negatives do occur". (Hefle, 9)

The power of the DBPCFC to diagnose true clinical reactivity to a food is very high. According to Dr. Wesley Burks, "When only objective signs and symptoms are scored, the results of blinded challenges are rarely equivocal." (Burks, 3)

Aventis strongly urges the FDA to take the advice of the SAP and to extend the investigative process of StarLink™ corn alleged allergic reactions to include the double blind, placebo controlled food challenge.

Conclusion

Although Aventis strongly believes that the existing toxicological data supports the conclusion that there is a "reasonable certainty of no harm" and concurs with the EPA's Scientific Advisory Panel that the probability of people experiencing significant allergic reactions to the Cry9C protein in StarLink™ is low, our desire is for the CDC/FDA to develop a robust scientific testing paradigm and avoid raising unnecessary concerns with respect to the safety of the US food supply. We believe incorporating these comments and suggestions into the investigative process ongoing at the CDC, FDA and EPA will enhance our progress towards these goals. Aventis is willing to transfer any technology,

protein, ELISA protocols to the FDA or independent laboratories to move this process forward in a scientifically defensible manner.

References

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Appendix 1 – Brief Regulatory History on StarLink Corn

Safety of the Cry9C Protein in StarLink™ Corn

The scientific evidence on the safety of StarLink™ corn includes data based on our knowledge of the genetic material and the plant pesticidal protein, Cry9C, expressed in the corn. The gene for the Cry9C protein comes from a non-allergenic common soil bacterium, *Bacillus thuringiensis*. The corn plant, into which the gene for the Cry9C protein was inserted, is rarely allergenic to humans. Expression of the gene for the Cry9C protein did not enhance the potential of corn to be allergenic, as demonstrated by no difference in reactivity to StarLink™ corn than to wild type non-transgenic corn in radioallergosorbent tests (RAST) performed with human sera from corn allergic patients (MRIDNumber 443844-05).

The Cry9C protein was not toxic upon single oral or repeated dietary administration to rats and has no linear amino acid sequence homology to any known human allergen or toxin (Oral LD50 > 3,760 mg/kg/day, MRID Number 442581-07; Acute intravenous LD50 > 0.3 mg/kg/day, MRID Number 447343-02; 30-day repeated dose toxicity test in rats: up to 328 mg/kg/day produced no adverse effects, no binding to villi or enterocytes lining GI tract crypts of both large and small intestines, MRID Number447343-03; MRID Numbers 443844-04, 442581-09). Additional confidence in the lack of allergenicity of the Cry9C protein was provided by radioallergosorbent tests performed with sera from individuals allergic to the well-known human food allergens, wheat, rice, buckwheat, soy, peanut, milk, eggs, and shrimp confirming that even individuals with pre-existing food allergies demonstrated no cross-reactivity to Cry9C (MRID Number 452464-01). The level of the Cry9C protein in whole corn grain, 0.0129%, is a very low level of total protein expression in the plant compared to most allergens which are present at 1-40% of the total plant protein (MRID Number 450257-01).

Protein Stability Not Toxicity Halted StarLink™ Corn Food Use Approval

The Cry9C protein is somewhat more stable than the other Bt Cry proteins already approved for food use. Cry9C does digest in simulated stomach fluids at pH of 1.2-1.5 within 30 – 60 minutes (within normal stomach emptying time) and does denature at temperatures likely to be encountered during cooking and processing (MRID Numbers 44734305, 44258108, 45114401, 445114402). The EPA did not grant human food use of StarLink™ corn due to uncertainty surrounding potential allergenicity of relatively stable proteins, even though protein stability alone is not sufficiently predictive of human allergenicity to classify the Cry9C protein, or any other protein, as a human allergen.

Expert Views on StarLink™ Corn Allergenicity Hazard to US Consumers

In characterizing the risk to the American population of allergic reactions to the Cry9C protein it must be noted that allergy experts from the Food Allergy Research and Resource Program at the University of Nebraska (Drs. Hefle and Taylor) emphasize that developing an allergy (allergic sensitization) requires multiple exposures over an extended period of time at sufficient levels to induce antibody formation. Both EPA- and Aventis-generated maximal theoretical worst case consumption calculations emphasize the extremely limited exposure potential for the Cry9C protein in the US diet.

The EPA's November 28, 2000 Scientific Advisory Panel concluded that there was a low probability that the likely levels of the Cry9C protein in the US diet were sufficient to cause significant allergic reactions in the exposed population. The SAP also concluded "the probability that StarLink™ corn has resulted in the sensitization of individuals to the Cry9C protein is low".

Aventis strongly believes that the minimal/low inherent allergenic potential of the Cry9C protein and the lack of multiple exposures over an extended period of time at sufficient quantities to sensitize people, support the conclusions that: it is highly improbable that there are individuals currently sensitized or "at risk" of allergic reactions from consumption of the Cry9C protein; there is a very low probability of ever becoming sensitized in the future to the Cry9C protein because the exposure potential is limited and diminishing with time as there is no StarLink™ corn being grown in 2001 and beyond; there is a very low probability of eliciting an adverse allergic reaction to the Cry9C protein.